Pine Chemicals Association
November 2004

VII. Robust Summaries of Data for Rosin Adducts and Adduct Salts

PHYSICO-CHEMICAL PROPERTY – WATER SOLUBILITY	
Test Substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 105, *Water Solubility*
Test Type	Water solubility
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, fumarated was tested for water solubility by weighing 0.5 g of the test item into an Erlenmeyer flask and adding 150 ml of Milli-Q water. The flasks were then shaken orbitally at 150 rpm at 30^0 C ± 1 ^{o}C for 72 h. A Milli-Q water blank was prepared in the same manner, without test material. At the end of the shaking period, the flasks were transferred to a water bath at 20 ^{0}C ± 1 ^{o}C for 24 h. The samples were then filtered if appropriate to remove undissolved material and allowed to sit overnight at ambient laboratory temperature. Samples were analyzed in triplicate. 100 ml of the supernatant/filtrate was adjusted to pH2 with phosphoric acid and extracted using preconditioned solid phase extraction cartridges, evaporated to residue, reconstituted in an internal standard solution, sonicated in an ultrasonic bath and derivatized with trimethylphenylammonium hydroxide (TMPAH). The test samples were then assayed by gas chromatography (GC) using flame ionization detection (FID). Behenic acid methyl ester was used as the internal standard.
Results	The water solubility of fumarated rosin is 9.0 mg/l at 20 ^{o}C.
Data Quality	Reliable without restrictions – Klimisch Code 1a
References	Dinwoodie, N.B. 2003. Determination of the Water Solubility of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters Report No. 24028, Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – WATER SOLUBILITY	
Test Substance	
Chemical Name	Rosin, maleated
CAS #	8050-28-0
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 105, Water Solubility
Test Type	Water solubility
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, maleated was tested for water solubility by weighing 0.5 g of the test item into an Erlenmeyer flask and adding 150 ml of Milli-Q water. The flasks were then shaken orbitally at 150 rpm at 30^0 C ± 1 ^{o}C for 72 h. A Milli-Q water blank was prepared in the same manner, without test material. At the end of the shaking period, the flasks were transferred to a water bath at 20 ^{0}C ± 1 ^{o}C for 24 h. The samples were then filtered if appropriate to remove undissolved material and allowed to sit overnight at ambient laboratory temperature. Samples were analyzed in triplicate. 100 ml of the supernatant/filtrate was adjusted to pH2 with phosphoric acid and extracted using preconditioned solid phase extraction cartridges, evaporated to residue, reconstituted in an internal standard solution, sonicated in an ultrasonic bath and derivatized with trimethylphenylammonium hydroxide (TMPAH). The test samples were then assayed by gas chromatography (GC) using flame ionization detection (FID). Behenic acid methyl ester was used as the internal standard.
Results	The water solubility of maleated rosin is 1.38 mg/l at 20 ^{0}C.
Data Quality	Reliable without restrictions – Klimisch Code 1a
References	Dinwoodie, N.B. 2003. Determination of the Water Solubility of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters Report No. 24028, Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – WATER SOLUBILITY	
Test Substance	
Chemical Name	Rosin, maleated/fumarated
CAS #	68554-16-5
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 105, Water Solubility
Test Type	Water solubility
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, maleated/fumarated was tested for water solubility by weighing 0.5 g of the test item into an Erlenmeyer flask and adding 150 ml of Milli-Q water. The flasks were then shaken orbitally at

	150 rpm at 30^0 C ± 1 ^{o}C for 72 h. A Milli-Q water blank was prepared in the same manner, without test material. At the end of the shaking period, the flasks were transferred to a water bath at 20 ^{0}C ± 1 ^{o}C for 24 h. The samples were then filtered if appropriate to remove undissolved material and allowed to sit overnight at ambient laboratory temperature. Samples were analyzed in triplicate. 100 ml of the supernatant/filtrate was adjusted to pH2 with phosphoric acid and extracted using preconditioned solid phase extraction cartridges, evaporated to residue, reconstituted in an internal standard solution, sonicated in an ultrasonic bath and derivatized with trimethylphenylammonium hydroxide (TMPAH). The test samples were then assayed by gas chromatography (GC) using flame ionization detection (FID). Behenic acid methyl ester was used as the internal standard.
Results	The water solubility of maleated/fumarated rosin is 0.58 mg/l at 20 ^{0}C.
Data Quality	Reliable without restrictions – Klimisch Code 1a
References	Dinwoodie, N.B. 2003. Determination of the Water Solubility of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters Report No. 24028, Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	1992
Test conditions	Rosin, fumarated and reference materials were dissolved in methanol and the solutions were analyzed by HPLC with UV detection using a mobile phase of 3:1 (v/v) methanol:buffer at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, fumarated had a partition coefficient range of 4.4 to 7.0.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Dybdahl, H.P. 1992. Determination of log P_{ow} for single componentsw in Fortified Rosin. Water Quality Institute, Horsholm, Denmark.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, maleated

CAS #	8050-28-0
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, maleated and reference materials were dissolved in methanol and the solutions were analyzed in duplicate by HPLC with Refractive Index (RI) and Photodiode Array (PDA) detection using a mobile phase of 25:75 (v/v) Milli-Q water/methanol at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, maleated had a partition coefficient range of 1.5 to 7.6.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Lightbody, S.M., Fisher, K. and Dinwoodie, N.B. 2003. Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Report No. 20977. Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, maleated/fumarated
CAS #	68554-16-5
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, maleated/fumarated and reference materials were dissolved in methanol and the solutions were analyzed in duplicate by HPLC with Refractive Index (RI) and Photodiode Array (PDA) detection using a mobile phase of 25:75 (v/v) Milli-Q water/methanol at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, maleated/fumarated had a partition coefficient range of 1.5 to 6.6.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Lightbody, S.M., Fisher, K. and Dinwoodie, N.B. 2003. Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Report No. 20977. Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, fumarated, sodium salt
CAS #	68201-59-2
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, fumarated, sodium salt and reference materials were dissolved in methanol and the solutions were analyzed in duplicate by HPLC with Refractive Index (RI) and Photodiode Array (PDA) detection using a mobile phase of 25:75 (v/v) Milli-Q water/methanol at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, fumarated, sodium salt had a partition coefficient range of 1.5 to 6.6.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Lightbody, S.M., Fisher, K. and Dinwoodie, N.B. 2003. Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Report No. 20977. Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, fumarated, potassium salt
CAS #	68649-83-2
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, fumarated, potassium salt and reference materials were dissolved in methanol and the solutions were analyzed in duplicate by HPLC with Refractive Index (RI) and Photodiode Array (PDA) detection using a mobile phase of 25:75 (v/v) Milli-Q water/methanol at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, fumarated, potassium salt had a partition coefficient range of 3.2 to 6.6.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Lightbody, S.M., Fisher, K. and Dinwoodie, N.B. 2003. Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Report No. 20977. Inveresk Research, Tranent, Scotland.

PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	Rosin, maleated, potassium salt
CAS #	85409-27-4
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 117, *"Partition Coefficient (n-Octanol/Water) High Performance Liquid Chromatograph (HPLC) Method"*
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	2003
Test conditions	Rosin, maleated, potassium salt and reference materials were dissolved in methanol and the solutions were analyzed in duplicate by HPLC with Refractive Index (RI) and Photodiode Array (PDA) detection using a mobile phase of 25:75 (v/v) Milli-Q water/methanol at pH 2. A mixture of seven materials with known log P_{ow} values was used for reference.
Results	At pH 2, rosin, maleated, potassium salt had a partition coefficient range of 1.4 to 7.9.
Data Quality	Reliable without restrictions – Klimisch Code 1a
Reference	Lightbody, S.M., Fisher, K. and Dinwoodie, N.B. 2003. Determination of the Partition Coefficient of Rosins, Rosin Salts, Rosin Adducts, Adduct Salts and Rosin Esters. Report No. 20977. Inveresk Research, Tranent, Scotland.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	Testing was conducted according to OECD Test Method 301 D, *"Ready Biodegradability: Closed Bottle Test"*
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1993
Contact time	28 days
Inoculum	Secondary effluent from Rungsted Treatment plant
Test conditions	Inoculum: Secondary effluent was collected from Rungsted Treatment plant in Horsholm. Concentration of test chemical: A stock solution of the test material (2 g/L) was prepared in demineralized water by ultra sonication for 5 minutes followed by magnetic stirring for 24 hours at 20^{o}C. The solution was filtered and, after determination of the chemical oxygen demand, it was used within the same day. Test Setup: Test medium was prepared by adding 1 mL each of four solutions (potassium phosphate, magnesium sulfate, calcium chloride, ferric chloride) to 1 liter of demineralized water, which was aerated to an initial oxygen concentration of approximately 9

	mg O_2/L and inoculated with 1 drop of secondary effluent per liter. The test article was added at 204 mg/L to a part of the inoculated test medium, equivalent to a chemical oxygen demand of 4.49 mg O_2/L. Sodium benzoate, the reference compound, was added at 2 mg/L to another part of the inoculated medium (to assess the activity of the inoculum), equivalent to a theoretical oxygen demand of 3.34 mg O_2/L. Both the test and reference articles (204 mg/L and 2 mg/L) were added to a third part of the inoculated medium (to assess possible inhibitory effects of the test article), at a theoretical oxygen demand of 7.83 mg O_2/L. Blank controls were prepared using the inoculated medium without test or reference materials. After the samples were prepared, the medium was transferred to calibrated respirometric bottles (BOD bottles), and placed in the dark at 20^{o}C. The study was performed in triplicate. Sampling frequency: Samples were collected for BOD analysis on days 0, 7, 14, 21, and 28. Controls: Yes. Method of calculating oxygen demand: Oxygen demand was calculated as the difference between the measured oxygen concentrations at time t and the start of the test. Biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand for the blank controls from the oxygen demand in the bottles containing test and reference compounds.
Results	
Degradation % after time	6.2% after 7 days and 15% after 28 days (test article); 59% after 7 days and 88% after 28 days (sodium benzoate)
Conclusions	The biological oxygen demand for fumarated rosin was 6.2 and 15% of the theoretical oxygen demand after 7 and 28 days, respectively. These data indicate that the test material is dominated by recalcitrant compounds. Fumarated rosin did not inhibit the respiratory activity of the inoculum. The inoculum had satisfactory activity as demonstrated by approximately 60% degradation within the 7 days using the reference compound.
Data Quality	Reliable without restrictions– Klimisch Code 1a
References	Madsen, T. 1993. Biodegradation of [fumarated] rosin. GLP Study No. 308067/476. Water Quality Institute, Horsholm, Denmark.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, maleated
CAS #	8050-16-5
Method	
Method/Guideline followed	Testing was conducted according to OECD (1992) 301B Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic

GLP (Y/N)	Y
Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from the Haddington Municipal Sewage Treatment Works
Test conditions	Inoculum: Activated sludge microorganisms were obtained from the Haddington Municipal Sewage Treatment Works. The solids content of the sludge was 4.2 g/l. Concentration of test chemical: The test material was used at a concentration of 20 mg DOC/L. Based on the percentage carbon content, a target weight of 53.2 mg of test material was weighed for direct addition to each appropriate bioreactor. Test Setup: Each test item bioreactor contained 1980 ml of mineral media, 20 ml of microbial inoculum and 53.2 mg of test item. The reference bioreactor contained 1911 ml of mineral media, 20 ml of microbial inoculum and 69 ml of reference material (sodium benzoate) stock. The control bioreactors each contained 1980 ml of mineral media and 20 ml of microbial inoculum. The toxicity control bioreactor contained 1911 ml of mineral media, 20 ml of microbial inoculum, 53.2 mg of test item and 69 ml reference material stock. Each bioreactor was connected to three traps, each trap containing 100 ml of 0.0125 M $Ba(OH)_2$. At trap collection, the trap closes to the bioreactor was taken for titration, the two remaining traps were moved closer to the bioreactor and a fresh trap was placed third in line from the bioreactor. Trap changes were conducted on days 1, 4, 6, 8, 11, 15, 18, 21, 25, and 29. Each sampled trap was titrated with a few drops of phenolphthalein indicator against 0.05M HCl. The pH was determined in each bioreactor on days 0, 28 and 29; if necessary, the pH on day 0 was adjusted to 7.2-7.8. Calculation of Results: The weight of CO_2 evolved was calculated from the titre. The actual titre for each batch of $Ba(OH)_2$ was used as the background titre. The mean titre for the test, reference and control vessels was calculated according to the following equation: Weight CO_2 produced (mg) = 1.1 x (background titre – ml HCl titrated) The net CO_2 production was then calculated by subtracting the control mean CO_2 production from the test and reference material mean CO_2 production values. The percentage biodegradation was calculated by comparing actual CO_2 evolved in test and reference vessels with the theoretical CO_2 evolution. For the test item this was calculated using the DOC addition rate: $\% \text{ degradation} = \frac{\text{Mg } CO_2 \text{ produced}}{\text{mg DOC added} \times 3.67^{*}} \times 100$

	* = where 3.67 is the conversion factor (44/12) for carbon to CO_2
Results Degradation % after time	0.34% after 29 days (test article); 75.09% after 28 days (sodium benzoate)
Conclusions	The test article was degraded <1 % after 28 days. Under the conditions of the OECD guidelines, it cannot be considered to be readily biodegradable.
Data Quality	Reliable without restrictions– Klimisch Code 1a
Reference	Kelly, C.R. 2002. Rosin, maleated, CAS No. 8050-28-0 Rosin, fumarated/maleated, CAS No. 68554-16-5. Determination of Ready Biodegradability by the Modified Sturm Test. Report No. 21511. Inveresk Research, Tranet, Scotland.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, fumarated/maleated
CAS #	68554-16-5
Method	
Method/Guideline followed	Testing was conducted according to OECD (1992) 301B Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from the Haddington Municipal Sewage Treatment Works
Test conditions	Inoculum: Activated sludge microorganisms were obtained from the Haddington Municipal Sewage Treatment Works. The solids content of the sludge was 4.2 g/l. Concentration of test chemical: The test material was used at a concentration of 20 mg DOC/L. Based on the percentage carbon content, a target weight of 53.5 mg of test material was weighed for direct addition to each appropriate bioreactor. Test Setup: Each test item bioreactor contained 1980 ml of mineral media, 20 ml of microbial inoculum and 53.5 mg of test item. The reference bioreactor contained 1911 ml of mineral media, 20 ml of microbial inoculum and 69 ml of reference material (sodium benzoate) stock. The control bioreactors each contained 1980 ml of mineral media and 20 ml of microbial inoculum. The toxicity control bioreactor contained 1911 ml of mineral media, 20 ml of microbial inoculum, 53.5 mg of test item and 69 ml reference material stock. Each bioreactor was connected to three traps, each trap containing 100 ml of 0.0125 M $Ba(OH)_2$. At trap collection, the trap closes to the bioreactor was taken for titration, the two remaining traps were moved closer to the bioreactor and a fresh

	trap was placed third in line from the bioreactor. Trap changes were conducted on days 1, 4, 6, 8, 11, 15, 18, 21, 25, and 29. Each sampled trap was titrated with a few drops of phenolphthalein indicator against 0.05M HCl. The pH was determined in each bioreactor on days 0, 28 and 29; if necessary, the pH on day 0 was adjusted to 7.2-7.8. Calculation of Results: The weight of CO_2 evolved was calculated from the titre. The actual titre for each batch of $Ba(OH)_2$ was used as the background titre. The mean titre for the test, reference and control vessels was calculated according to the following equation: Weight CO_2 produced (mg) = 1.1 x (background titre – ml HCl titrated) The net CO_2 production was then calculated by subtracting the control mean CO_2 production from the test and reference material mean CO_2 production values. The percentage biodegradation was calculated by comparing actual CO_2 evolved in test and reference vessels with the theoretical CO_2 evolution. For the test item this was calculated using the DOC addition rate: $$\% \text{ degradation} = \frac{\text{Mg } CO_2 \text{ produced}}{\text{mg DOC added} \times 3.67^{*}} \times 100$$ * = where 3.67 is the conversion factor (44/12) for carbon to CO_2
Results Degradation % after time	18.92% after 29 days (test article); 75.09% after 28 days (sodium benzoate)
Conclusions	The test article was degraded 19% after 28 days. Under the conditions of the OECD guidelines, it cannot be considered to be readily biodegradable.
Data Quality	Reliable without restrictions– Klimisch Code 1a
Reference	Kelly, C.R. 2002. Rosin, maleated, CAS No. 8050-28-0 Rosin, fumarated/maleated, CAS No. 68554-16-5. Determination of Ready Biodegradability by the Modified Sturm Test. Report No. 21511. Inveresk Research, Tranet, Scotland.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, fumarated, sodium salt
CAS #	68201-59-2
Method	
Method/Guideline followed	Testing was conducted according to OECD (1992) 302B *"Modified Zahn-Wellens Test"*
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y

Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from the Haddington Municipal Sewage Treatment Works
Test conditions	Inoculum: Activated sludge microorganisms were obtained from the Haddington Municipal Sewage Treatment Works. The solids content of the sludge was 3.8 g/l. Concentration of test chemical: The test material was used at a concentration of 200 mg DOC/L equivalent to 5270 mg of rosin, fumarated sodium salt per 2.5 liter bioreactor based on percentage total carbon content. To prepare the reference material (aniline) 8.09 ml was added to 1000 ml DI water and sonicated for *ca* 30 min. to aid dissolution; from this stock 125 ml aliquots were added to each appropriate bioreactor at 400 mg/DOC/l equivalent to 125 ml of a 6400 mg/DOC/l solution per 2 liter bioreactor. Test Setup: The activated sludge was introduced to the test system at a ratio of 2.5:1 sludge solids/l to test item DOC/l which required the addition of 263 ml of 3.8 g/l sludge to each bioreactor. A total of five bioreactors were used. Each bioreactor had a final volume of 2000 ml; the control bioreactors each contained 250 ml sludge and 1750 ml of mineral medium; the reference bioreactor contained 250 ml sludge, 125 ml of aniline stock solution and 1625 of mineral medium; the two test item bioreactors each contained 250 ml sludge, the appropriate weight of test item and 1750 ml of mineral medium; the toxicity control bioreactor contained 250 ml sludge, the appropriate weight of test item, 125 ml aniline stock and 1625 ml mineral medium. The test was conducted over 28 days. DOC measurements were conducted on duplicate samples 3 h after test initiation, and on Days 14 and 28. Sampling Procedure: Prior to each sampling point the liquid in each vessel was replenished to its starting level. The pH and dissolved oxygen concentration were recorded. If necessary the pH was adjusted to 6.5-8.0 using H_2SO_4 as appropriate. A *ca* 25 ml sample was extracted from each vessel using a syringe and allowed to settle after which it was passed through a 0.45um filter for DOC analysis. Determination of DOC, total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) were determined. Calculation of Results: All measurements were conducted on duplicate samples. Dissolved organic carbon (DOC) values were calculated as follows: DOC = TC – IC The percentage degradation (Dt) at each timepoint was calculated using mean DOC measurements from the duplicate samples, using the following equation: (Ct -Cb)

	Dt = (1 - ----------) x 100 (Ca – Cba) Where: Ct = mean DOC concentration in test/reference at time t Cb = mean DOC concentration in controls at time t Ca = mean DOC concentration in test/reference at 3 h ± 0.5 h Cba = mean DOC concentration in controls at 3 h ± 0.5 h
Results Degradation % after time	Rosin, fumarated sodium salt reached 48% degradation by Day 28; the reference material reached 97.1% degradation by Day 14. Based on total carbon content this was equivalent to 35.6% of the whole test item.
Conclusions	The test article was degraded 48% after 28 days under the conditions of the test.
Data Quality	Reliable without restrictions– Klimisch Code 1a
Reference	Kelly, C.R. 2002. Rosin, potassium salt CAS No. 61790-50-9; Rosin, fumarated, sodium salt, CAS No. 68201-59-2; Rosin, fumarated, potassium salt, CAS No. 68649-83-2; Rosin, maleated, potassium salt, CAS No. 85409-27-4, Determination of Inherent Biodegradability by the Modified Zahn-Wellens Test. Report No. 21487. Inveresk Research, Tranent, Scotland.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, fumarated, potassium salt
CAS #	68649-83-2
Method	
Method/Guideline followed	Testing was conducted according to OECD (1992) 302B *"Modified Zahn-Wellens Test"*
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from the Haddington Municipal Sewage Treatment Works
Test conditions	Inoculum: Activated sludge microorganisms were obtained from the Haddington Municipal Sewage Treatment Works. The solids content of the sludge was 2.8 g/l. Concentration of test chemical: The test material was used at a concentration of 200 mg DOC/L equivalent to 4836 mg of rosin, fumarated potassium salt per 2.5 liter bioreactor based on percentage total carbon content. To prepare the reference material (aniline) 8.09 ml was added to 1000 ml DI water and sonicated for *ca* 30 min. to aid dissolution; from this stock 125 ml aliquots were added to each appropriate bioreactor at 400 mg/DOC/l equivalent to 125 ml of a 6400 mg/DOC/l solution per 2 liter bioreactor.

	Test Setup: The activated sludge was introduced to the test system at a ratio of 2.5:1 sludge solids/l to test item DOC/l which required the addition of 357 ml of 2.8 g/l sludge to each bioreactor. A total of five bioreactors were used. Each bioreactor had a final volume of 2000 ml; the control bioreactors each contained 250 ml sludge and 1750 ml of mineral medium; the reference bioreactor contained 250 ml sludge, 125 ml of aniline stock solution and 1625 of mineral medium; the two test item bioreactors each contained 250 ml sludge, the appropriate weight of test item and 1750 ml of mineral medium; the toxicity control bioreactor contained 250 ml sludge, the appropriate weight of test item, 125 ml aniline stock and 1625 ml mineral medium. The test was conducted over 28 days. DOC measurements were conducted on duplicate samples 3 h after test initiation, and on Days 14 and 28. Sampling Procedure: Prior to each sampling point the liquid in each vessel was replenished to its starting level. The pH and dissolved oxygen concentration were recorded. If necessary the pH was adjusted to 6.5-8.0 using H_2SO_4 as appropriate. A *ca* 25 ml sample was extracted from each vessel using a syringe and allowed to settle after which it was passed through a 0.45um filter for DOC analysis. Determination of DOC, total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) were determined. Calculation of Results: All measurements were conducted on duplicate samples. Dissolved organic carbon (DOC) values were calculated as follows: $DOC = TC - IC$ The percentage degradation (Dt) at each timepoint was calculated using mean DOC measurements from the duplicate samples, using the following equation: $$Dt = \left(1 - \frac{Ct - Cb}{Ca - Cba}\right) \times 100$$ Where: Ct = mean DOC concentration in test/reference at time t Cb = mean DOC concentration in controls at time t Ca = mean DOC concentration in test/reference at 3 h ± 0.5 h Cba = mean DOC concentration in controls at 3 h ± 0.5 h
Results Degradation % after time	Rosin, fumarated potassium salt reached 50.5% degradation by Day 28; the reference material reached 97.1% degradation by Day 14. Based on total carbon content this was equivalent to 35.3% of the whole test item.
Conclusions	The test article was degraded 50.5% after 28 days under the conditions of the test.
Data Quality	Reliable without restrictions– Klimisch Code 1a

Reference	Kelly, C.R. 2002. Rosin, potassium salt CAS No. 61790-50-9; Rosin, fumarated, sodium salt, CAS No. 68201-59-2; Rosin, fumarated, potassium salt, CAS No. 68649-83-2; Rosin, maleated, potassium salt, CAS No. 85409-27-4, Determination of Inherent Biodegradability by the Modified Zahn-Wellens Test. Report No. 21487. Inveresk Research, Tranent, Scotland.

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	Rosin, maleated, potassium salt
CAS #	85409-27-4
Method	
Method/Guideline followed	Testing was conducted according to OECD (1992) 302B *"Modified Zahn-Wellens Test"*
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	2002
Contact time	28 days
Inoculum	Activated sludge from the Haddington Municipal Sewage Treatment Works
Test conditions	Inoculum: Activated sludge microorganisms were obtained from the Haddington Municipal Sewage Treatment Works. The solids content of the sludge was 2.8 g/l. Concentration of test chemical: The test material was used at a concentration of 200 mg DOC/L equivalent to 4836 mg of rosin, maleated potassium salt per 2.5 liter bioreactor based on percentage total carbon content. To prepare the reference material (aniline) 8.09 ml was added to 1000 ml DI water and sonicated for *ca* 30 min. to aid dissolution; from this stock 125 ml aliquots were added to each appropriate bioreactor at 400 mg/DOC/l equivalent to 125 ml of a 6400 mg/DOC/l solution per 2 liter bioreactor. Test Setup: The activated sludge was introduced to the test system at a ratio of 2.5:1 sludge solids/l to test item DOC/l which required the addition of 357 ml of 2.8 g/l sludge to each bioreactor. A total of five bioreactors were used. Each bioreactor had a final volume of 2000 ml; the control bioreactors each contained 250 ml sludge and 1750 ml of mineral medium; the reference bioreactor contained 250 ml sludge, 125 ml of aniline stock solution and 1625 of mineral medium; the two test item bioreactors each contained 250 ml sludge, the appropriate weight of test item and 1750 ml of mineral medium; the toxicity control bioreactor contained 250 ml sludge, the appropriate weight of test item, 125 ml aniline stock and 1625 ml mineral medium. The test was conducted over 28 days. DOC measurements were conducted on duplicate samples 3 h after test initiation, and on Days 14 and 28. Sampling Procedure: Prior to each sampling point the liquid in

	each vessel was replenished to its starting level. The pH and dissolved oxygen concentration were recorded. If necessary the pH was adjusted to 6.5-8.0 using H_2SO_4 as appropriate. A *ca* 25 ml sample was extracted from each vessel using a syringe and allowed to settle after which it was passed through a 0.45um filter for DOC analysis. Determination of DOC, total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) were determined. Calculation of Results: All measurements were conducted on duplicate samples. Dissolved organic carbon (DOC) values were calculated as follows: DOC = TC – IC The percentage degradation (Dt) at each timepoint was calculated using mean DOC measurements from the duplicate samples, using the following equation: $Dt = \left(1 - \frac{Ct - Cb}{Ca - Cba}\right) \times 100$ Where: Ct = mean DOC concentration in test/reference at time t Cb = mean DOC concentration in controls at time t Ca = mean DOC concentration in test/reference at 3 h ± 0.5 h Cba = mean DOC concentration in controls at 3 h ± 0.5 h
Results Degradation % after time	Rosin, maleated potassium salt reached 59.2% degradation by Day 28; the reference material reached 98.6% degradation by Day 14. Based on total carbon content this was equivalent to 52.6% of the whole test item.
Conclusions	The test article was degraded 59.2% after 28 days under the conditions of the test.
Data Quality	Reliable without restrictions– Klimisch Code 1a
Reference	Kelly, C.R. 2002. Rosin, potassium salt CAS No. 61790-50-9; Rosin, fumarated, sodium salt, CAS No. 68201-59-2; Rosin, fumarated, potassium salt, CAS No. 68649-83-2; Rosin, maleated, potassium salt, CAS No. 85409-27-4, Determination of Inherent Biodegradability by the Modified Zahn-Wellens Test. Report No. 21487. Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ACUTE TOXICITY TO FISH	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Method 203, *"Testing of Chemicals, Fish Acute Toxicity Test"* and following procedures in OECD (2000) Series on Testing and Assessment, No. 23, *"Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures."*

Year	2002
GLP (Y/N)	Y
System of testing	Fathead minnows (*Pimephales promelas)* under static conditions.
Concentration	0, 1, 10, 100 and 1000 mg/l
Results	The 96 hr LL_{50} was > 1000 mg/l the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Detailed Summary	Rosin, fumarated was tested in fathead minnows under static conditions to determine acute toxicity. Water accommodated fractions (WAF) were prepared using the same conditions as those used to determine the water solubility of this substance. Appropriate weights of fumarated rosin were added to a stirring medium in glass vessels which were sealed to avoid loss of volatile fractions. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. The WAF was then removed via a glass siphon taking care not to remove undissolved material at the top or bottom of the water column. The test organisms were exposed to this WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions, but to reduce exposure to the test organisms to insoluble fractions. A control medium without the addition of the test item was stirred and extracted in an identical ways as the treated media. The effects of both filtering and adjusting pH were investigated in a range finding test using the highest loading rate (i.e., 1000 mg/l). Because no mortality or other effects were observed in the range finding tests, a definitive-limit test was conducted at the maximum loading rate of 1000 mg/l. The 96 hr LL_{50} was > 1000 mg/l, the highest loading rate tested. The No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Rosin, fumarated, CAS No. 65997-04-8 Determination of Acute Toxicity (LL_{50}) to Fathead Minnows (96 h, Static). Report Number 20934. Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ACUTE TOXICITY TO DAPHNIA	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Method 202, Part 1 *"Testing of Chemicals, Daphnia sp. Acute Immobilization Test"* and following procedures in OECD (2000) Series on Testing and Assessment, No. 23, *"Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures."*
Year	2002
GLP (Y/N)	Y
System of testing	*Daphnia magna* (water fleas) under static conditions.
Concentration	0, 10, 100, and 1000 mg/l (range-finding test) 1000 mg/l (definitive test)

Results	The 48 hr EL_{50} was > 1000 mg/l; the No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Detailed Summary	Fumarated rosin was tested in daphnia under static conditions to determine the acute toxicity. Water accommodated fractions (WAF) were prepared using the same conditions as those used to determine the water solubility of this substance. Appropriate weights of fumarated rosin were added to a stirring medium in glass vessels which were sealed to avoid loss of volatile fractions. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. The WAF was then removed via a glass siphon taking care not to remove undissolved material at the top or bottom of the water column. The test organisms were exposed to this WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions, but to reduce exposure to the test organisms to insoluble fractions. A control medium without the addition of the test item was stirred and extracted in an identical ways as the treated media. The effects of both filtering and adjusting pH were investigated in a range finding test. Because there was no mortality in the range finding test at any WAF, a definitive limit test was conducted with an unfiltered WAF at the maximum loading rate of 1000 mg/l. The 48 hr EL_{50} was >1000 mg/l and the No Observed Effect Loading Rate ($NOEL_r$) was 1000 mg/l.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Rosin, fumarated CAS No. 65997-04-8 Determination of Acute Toxicity (EL_{50}) to Daphnia (48 h, Static). Report Number 21149. Inveresk Research, Tranent, Scotland.

ECOTOXICITY – ALGA, GROWTH INHIBITION	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Method 201, *"Testing of Chemicals, Alga, Growrh Inhibition Test"* and following procedures in OECD (2000) Series on Testing and Assessment, No. 23, *"Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures."*
Year	2002
GLP (Y/N)	Y
System of testing	Green alga (*Selenastrum capriconutum)* growth inhibition.
Concentration	0, 1, 10, 100 and 1000 mg/l (range finding test) 1000 mg/l (definitive test)
Results	The 72 hr EL_{50} for area under growth curve (AUC) and Average Specific Growth Rate (0-72h) was > 1000 mg/l. The No Observed Effect Loading Rate ($NOEL_r$) for Average Specific Growth Rate and AUC was 1000 mg/l.
Detailed Summary	Fumarated rosin was tested in alga to determine the median effective loading (EL_{50}) for growth inhibition. Water accommodated fractions (WAF) were prepared using the same conditions as those used to determine the water solubility of this

	substance. Appropriate weights of fumarated rosin were added to a stirring medium in glass vessels which were sealed to avoid loss of volatile fractions. Using magnetic stirrers, the stirring speed was adjusted to give a stirring vortex 5-10% of the water column. After a stirring period of approximately 48 hr. the test solutions were allowed to settle for ca hour. The WAF was then removed via a glass siphon taking care not to remove undissolved material at the top or bottom of the water column. The test organisms were exposed to this WAF. This procedure was adopted to maximize the solubility of the test item under specific test exposure conditions, but to reduce exposure to the test organisms to insoluble fractions. A control medium without the addition of the test item was stirred and extracted in an identical ways as the treated media. The effects of both filtering and adjusting pH were investigated in a range finding test at the highest loading rate. Because there was no inhibition of algal growth in the range finding test in any test groups, a definitive limit test was conducted at 1000 mg/l with algal cell concentrations recorded after 1, 24, 48 and 762 hrs. This test was conducted using an unfiltered WAF with no pH adjustment. As no effects or inhibition was observed the 72 hr EL_{50} was > 1000 mg/l for area under growth curve (AUC) and Average Specific Growth Rate (0-72h). Consequently, the No Observed Effect Loading Rate ($NOEL_r$) for AUC and Average Specific Growth Rate is 1000 mg/l.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Kelly, C. 2002. Rosin, fumarated CAS No. 65997-04-8 Alga, Growth Inhibition Test (72 h, EL_{50}). Report Number 21012. Inveresk Research, Tranent, Scotland.

ACUTE TOXICITY – ORAL	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	Test procedure was OECD Test Method 425 *"Acute Oral Toxicity – Up-and-Down Procedure"*
GLP (Y/N)	Y
Year (Study Performed)	2002
Species	Rat
Strain	Sprague-Dawley
Route of administration	Oral
Dose levels	2000 mg/kg
Sex and number/group	5 female rats
Frequency of treatment	Single oral gavage
Duration of test	14 day observation post-treatment
Control group (Y/N)	N
Result	
Acute Oral LD_{50}	>2,000 mg/kg
Detailed Summary	The initial dose level was 2000 mg/kg as this is the dose level suggested by the OECD Guideline. One female animal was dosed with 2000 mg/kg. Since this animal survived, 4 additional

	animals were dosed sequentially at 2000 mg/kg so that a total of 5 animals were tested. The test item was dissolved in corn oil and administered orally in a single dose, by means of a gavage, followed by a 14 day observation period. A dose volume of 10 ml/kg was used for the first animal and increased to 20 ml/kg for subsequent animals due to difficulty gavaging with the initial volume. All animals were examined for reaction to treatment. The onset, intensity and duration of any signs were recorded. Clinical observations were conducted frequently after dosing on Day 1and daily thereafter until Day 15. There were no mortalities or adverse clinical signs noted during the observation period. Body weight gain was considered to have been satisfactory. No findings were noted at necropsy. Following a single oral administration of rosin, fumarated to Sprague-Dawley rats, the median lethal dose (LD_{50}) was estimated to be > 2000 mg/kg.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Hutchinson, A.M.K. 2002. Rosin, fumarated(CAS No. 65997-04-8) Acute Oral Toxicity (Up-and-Down Procedure) Test in Rats. Report No. 22036. Inveresk Research, Tranent, Scotland.

ACUTE TOXICITY – ORAL	
Test substance	
Chemical Name	Rosin, maleated
CAS #	8050-28-0
Method	
Method/Guideline followed	Test procedure was OECD Test Method 425 *"Acute Oral Toxicity – Up-and-Down Procedure"*
GLP (Y/N)	Y
Year (Study Performed)	2002
Species	Rat
Strain	Sprague-Dawley
Route of administration	Oral
Dose levels	2000 mg/kg
Sex and number/group	5 female rats
Frequency of treatment	Single oral gavage
Duration of test	14 day observation post-treatment
Control group (Y/N)	N
Result	
Acute Oral LD_{50}	>2,000 mg/kg
Detailed Summary	The initial dose level was 2000 mg/kg as this is the dose level suggested by the OECD Guideline. One female animal was dosed with 2000 mg/kg. Since this animal survived 4 additional animals were dosed sequentially at 2000 mg/kg so that a total of 5 animals were tested. The test item was dissolved in corn oil and administered orally in a single dose, by means of a gavage, followed by a 14 day observation period. A constant dose volume of 10 ml/kg was used. All animals were examined for reaction to treatment. The onset, intensity and duration of any signs were recorded. Clinical observations were conducted frequently after dosing on Day

	1and daily thereafter until Day 15. There were no mortalities or adverse clinical signs noted during the observation period. Body weight gain was considered to have been satisfactory. No findings were noted at necropsy. Following a single oral administration of rosin, maleated to Sprague-Dawley rats, the median lethal dose (LD_{50}) was estimated to be > 2000 mg/kg.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Hutchinson, A.M.K. 2002. Rosin, maleated (CAS No. 8050-28-0) Acute Oral Toxicity (Up-and-Down Procedure) Test in Rats. Report No. 22018. Inveresk Research, Tranent, Scotland.

REPEAT DOSE TOXICITY WITH REPRODUCTIVE/DEVELOPMENTAL TOXICITY SCREENING TEST	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Guideline 422, *"Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test."*
GLP (Y/N)	Y
Year (Study Performed)	2002
Species	Rat
Strain	Sprague-Dawley
Route of administration	Oral via diet
Dose levels	0, 1000, 3000 and 10000 ppm.
Sex and number/group	40 males and 40 females
Frequency of treatment	Males were treated for at least 4 weeks overall, starting from 2 weeks prior to mating until termination; females were treated for 2 weeks prior to mating, then through mating until termination after Day 4 of lactation.
Duration of test	4 weeks
Control group (Y/N)	Y
Result	
Parental NOEL Reproductive/developmental NOEL	1000 ppm (91 mg/kg/day) 3000 ppm (260 mg/kg/day)
Detailed Summary	Four groups of 10 male and 10 female Sprague-Dawley rats received the fumarated rosin *via* the diet at concentrations of 0, 1000, 3000 and 10000 ppm. The males were dosed for at least 4 weeks, starting from 2 weeks prior to mating. The females were dosed from 2 weeks prior to mating until at least Day 6 of lactation. The animals were monitored for clinical signs, body weight, food consumption, mating and litter performance. Blood samples were taken from 5 males and 5 females per group for laboratory investigations. Males were sampled during Week 5: females were sampled on Day 6 of lactation. All animals were subjected to necropsy, which included weighing of major organs. Histopathology was conducted on tissues from 5 males from Control and High dose, and 7 females from the Control and 8 females from the High dose.

	Clinical observations associated with treatment were abnormal colored urine at all treatment levels and soft fecal output at 3000 and 10000 ppm. At 3000 and 10000 ppm, there was a decrease in mean body weight gain and food consumption in both sexes; this was only significant at the highest dose. There were no obvious effects on body weight gain or food consumption in animals treated at 1000 ppm. At 10000 ppm, there were slight increases in the number of nights to a positive mating sign, and in the duration of gestation. Additionally, the mean number of implants per pregnancy was slightly decreased with a subsequent reduction in litter size and litter weight; this effect was most likely due to decreased food consumption and weight gain. There were no obvious effects of treatment on mating performance, duration of gestation, litter size and pup weight in animals treated at 1000 and 3000 ppm. Total bilirubin was significantly increased in both sexes treated at 10000 ppm and there was a slight decrease in hemoglobin, red blood cell count and hematocrit in males only. At 10000 ppm, in females, there was a significant decrease in adrenal gland weight, which still remained evident after covariance adjustment. Histological examination revealed thymic atrophy in females treated at 10000 ppm, but given the reduced body weight during pregnancy, the findings were considered likely to reflect the physiological status of the animals. Under the conditions of this study, maternal toxicity (i.e., decreased body weight gain and food consumption) was observed at levels of 3000 and 10000 ppm. However, there were no clear effects of toxicity at 1000 ppm. Therefore the parental No Observed Effect Level (NOEL) was considered to be 1000 ppm (91 mg/kg). For reproductive/developmental parameters the NOEL was considered to be 3000 ppm (260 mg/kg).
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Clubb, S. and Sutherland, J.R. 2002. Rosin, fumarated (CAS No. 68997-04-8) Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test. Report Number 21890. Inveresk Research, Tranent, Scotland.

IN VITRO GENETIC TOXICITY	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Method 471, *"Bacterial Reverse Mutation Test"*
Year	2002
GLP (Y/N)	Y
System of testing	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 *E. coli* WP2*uvr*A
Concentration	17, 50, 167, 500, 1667, and 5000 µg/plate
Metabolic activation	With and without addition of S-9 fraction from Aroclor 1254 treated Sprague-Dawley rats.

Results	Fumarated rosin was non-mutagenic with or without metabolic activation
Detailed Summary	Fumarated rosin was tested in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* WP2*uvr*A for mutagenic activity. The test article was tested at concentrations of 17, 50, 167, 500, 1667, and 5000 µg/plate with and without metabolic activation with S9 fraction from Aroclor 1254-treated adult male Fisher rats. Positive controls not requiring metabolic activation included N-ethyl-N-nitro-N-nitrosoguanidine (EENG), 9-aminoacridine, 2-nitrofluorene, and sodium azide; the positive control requiring metabolic activation was 2-aminoanthracene. No increases in mutation frequency were reported at any concentration of fumarated rosin with or without metabolic activation. Fumarated rosin was not mutagenic in this assay to *S. thyphimurium* or *E. coli* either with or without metabolic activation to a maximum limit of 5000 µg/plate.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Stevenson, F.M. 2001. Fumarated Rosin, CAS No. 65997-04-8 Testing for Mutagenic Activity with *Salmonella Typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2*uvr*A. Report Number 20912. Inveresk Research, Tranent, Scotland.

IN VITRO GENETIC TOXICITY	
Test substance	
Chemical Name	Rosin, fumarated
CAS #	65997-04-8
Method	
Method/Guideline followed	OECD Test Method 473, *"Chromosomal Aberration Assay with Chinese Hamster Ovary Cells in vitro."*
Year	2002
GLP (Y/N)	Y
System of testing	Chinese Hamster Ovary (CHO) cells *in vitro*
Concentrations of test material selected for assessment of chromosomal aberrations	Test 1: With S9 mix: 10, 20, 40, 60 and 80 ug/ml Test 2: With S9 mix: 5, 10, 20, 30, 40 and 50 ug/ml Test 1: Without S9 mix: 20, 40, 60 and 80 ug/ml Test 2: Without S9 mix: 40, 60, 80, 95, 110 and 120 ug/ml (24 h harvest) Test 2: Without S9 mix: 20, 40, 80, 120, 160 and 200 ug/ml (48 h harvest)
Metabolic activation	With and without addition of S9 fraction from Aroclor 1254-treated adult male Fisher rats.
Results	Fumarated rosin was non-clastogenic with or without S9 mix; a slight increase in polyploidy was induced in the absence of S9 at a concentration level that was deemed overtly toxic to the cells.
Detailed Summary	Fumarated rosin was tested in Chinese hamster ovary (CHO) cells for clastogenic activity both with and with metabolic activation with rat liver S9 mix. The test article was tested with metabolic activation with S9 mix at concentrations of 10, 20, 40, 60 and 80 ug/ml (Test 1) and in Test 2 at 40, 60, 80, 95, 110 and 120 ug/ml without metabolic activation with S9 mix and at concentrations of 5, 10, 20, 30, 40, and 50 ug/ml with S9 mix. The positive controls requiring and not requiring metabolic

	activation were cyclophosphamide (CPH) and methanesulphonate (MMS), respectively. Treatments with test item or controls were performed on duplicate cell cultures. Two slides per culture up to 50 metaphase cells per slide were examined. A dose level was considered to be toxic if the cell count was reduced to less than 50% of the mean vehicle control values or if consistent evidence of changes to cell morphology was observed. There was no evidence that fumarated rosin induced structural chromosomal aberrations either in the presence of absence of S9 mix within the 95% confidence limits of the historical negative control data, even when assessed into the toxic range. However, in the absence of S9 mix in cultures harvested at 48 h there was a slight increase in polyploidy cells at a concentration level (120 µg/ml) that was judged overtly toxic to the cells.
Data Quality	Valid without restriction – Klimisch Code 1a
Reference	Murie, E. 2002. Rosin, fumarated, CAS No. 65997-04-8 Chromosomal Aberration Assay with Chinese Hamster Ovary Cells in vitro (Complying with EC (Annex V) and OECD 473 Guidelines). Report Number 21092. Inveresk Research, Tranent, Scotland.